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HISTOPATHOLOGY OF CORONARY ARTERY AND SEROLOGY OF SERUM LIPIDS IN RABBITS FED DIETS OF COWPEA, SOYABEAN, CASSAVA AND MELON

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ABSTRACT
A study carried out in the Teaching and Research Farm of Rivers State University of Science and Technology, Nkpolu, Port Harcourt, demonstrated the atherogenic effects of cowpea (*Vigna unguiculata*), melon (*Citrillus vulgaris*), soyabean (*Glycine max*) and cassava (*Manihot utilissima*) feedstuffs using Chinchilla grower rabbits. The rabbits were randomly allotted to five treatment groups, namely, T_1 (corn), control, T_2 (Cowpea), T_3 (Melon), T4 (Soyabean) and T_5 (Cassava). Each treatment group had six replicate units comprising three males and three females, in a Randomized Complete Block Design (RCBD). The rabbits were fed nonisonitrogenous and nonisocaloric diets *ad libitum* for 12 weeks, housed individually in hutches. At the end of the 12 weeks, 20 rabbits were slaughtered to determine the effect of the treatments (feedstuffs) and histology of the coronary artery. Thereafter the remaining 10 rabbits were fed garlic (*Allium sativum*) at the rate of 2% dietary inclusion (20g/kg feed) for six weeks, and the rabbits were slaughtered to determine the effects of feeding garlic on the same aforementioned parameters. Histophotomicrographic characterization of the coronary arteries of the heart was also carried out. It was observed that there were significant differences ($P<0.05$) in serum Cholesterol, HDL and LDL levels in the blood of rabbits before and after feeding of garlic. Histophotomiciographic characterization showed that cassava and melon caused thickening of the arterial wall of the coronary arteries of the heart which was indicative of atherosclerosis while garlic feeding produced a significant regression of the erstwhile observed thickness. It was therefore concluded that cassava and melon are atherogenic but cowpea slightly so, and that garlic feeding caused a regression of the atherogenesis produced by these feedstuffs while soyabean showed no atherogenic effect.

KEYWORDS: Atherogens, rabbits, histophotomicrographic characterization, feedstuffs, atherosclerosis, regression.

INTRODUCTION

Feedstuffs which on consumption or incorporation could lead to an increase in the level of cholesterol production by the body (liver) are referred to as atherogens. A high level of serum cholesterol especially low density lipoprotein cholesterol (LDL – Cholesterol), the "bad-cholesterol", leads to atherosclerosis which is a condition in which an artery wall thickens as a result of build-up of fatty materials such as cholesterol, in a chronic inflammatory response due to the accumulation of macrophages resulting to a hardening and in-elasticity of the arteries (Maton et al, 1993). Atherosclerosis eventually produces two main problems. First, the atheromantous plaques eventually lead to plaque ruptures and clots inside the artery lumen. The clots heal and usually shrink but leave behind a narrowing (stenosis) of the artery both locally and in smaller downstream branches, or a complete closure, and therefore, an insufficient blood supply to the tissues. When the enlargement process is excessive, then a net aneurysm results (Nissen et al, 2006). Most commonly, soft plaque suddenly ruptures, causing the formation of a thrombus (thrombosis) that will rapidly slow or stop blood flow, leading to a sudden death of the tissues fed by the artery. This is called an infarction and is usually catastrophic. One of the most commonly recognized is called "coronary thrombosis" of a coronary artery, causing myocardial infarction (heart attack). Similarly, if artery to the brain (carotid artery) is involved, "stroke" results. Other scenarios of advanced or chronic atherosclerosis include claudication, which is limping or lameness, and similar disabilities of the hands or legs, and the internal organs. In this study, feedstuffs which people normally resort to as alternatives to the risk-prone or cholesterol-prone entities such as cowpea and soyabean, will be investigated. Also to be investigated for atherogenesis is melon, a common delicacy on most Nigerian menu, and cassava (garri) a common Nigerian feedstuff which has recently gained international popularity and is now being exported. Garlic will be used in this study to investigate regression of plaques and other indices that support regression of atherosclerosis.

The objectives of this study therefore are to investigate the presence of cholesterolic plaques in the coronary artery of the heart, to assess the atherogenic effect of these feedstuffs in rabbits through histophotomicrographic plating and regression effect by garlic.

MATERIALS AND METHODS

Thirty (30) Chinchilla grower rabbits were given the following dietary treatments (rations) in-order to

determine their atherogenic effects (ability to cause atherosclerosis):

(1) T_1 (Corn meal or *Zea mays*), control
(2) T_2 (Cowpea or *Vigna unguiculata*), commonly called black eyed peas or beans
(3) T_3 (Melon meal or *Citrullus vulgaris*), commonly called egusi
(4) T_4 (Soyabean or *Glycine max*), peeled and ground (full fat).
(5) T_5 (Cassava or *Manihot utilissima*), as fried cassava meal commonly called garri.

Each of the five treatments (T_1,T_2,T_3,T_4 and T_5) comprised six rabbits in six replicates of three males and three females, giving a total of 30 rabbits. The rabbits were fed individually in hutches with the test diets or treatments (cowpea, melon, soyabean, cassava and the control, maize) *ad libitum* for 12 weeks in the first phase of the study. Daily feed intake was recorded by subtracting the left-over feed from that initially fed.

At the end of the first 12 weeks, 20 rabbits (10 males and 10 females) were weighed and then slaughtered by cervical severing and blood samples of 5ml each were collected in "test tubes without anticoagulant" (a) Determination of total serum lipids (Cholesterol, LDL, HDL and TG). (b) Determination of the magnitude of internal thickening of lipids (plaques) in the coronary arteries through histophotomicrographic plating. The coronary arteries of the heart were also collected and preserved in 10% Formal-Saline for histological characterization.

Thereafter, the remaining 10 rabbits were fed the control (corn meal) diet with the incorporation of 2% garlic (*Allium sativum*) powder, also called *alivosar*, that is, at the rate of 20g/kg feed. They were fed for six (6) weeks. The rabbits were slaughtered at the end of the six weeks in the same manner. Blood and the coronary arteries of the heart were collected again for the determination of the parameters mentioned above. The lipid metabolites measured from the experimental blood samples were subjected to Analysis of Variance (ANOVA) and means separated by Ducan Multiple Range Test (Gill, 1978), while the histopathological, histophotomicrographic plates were contrasted and characterized under the light microscope to determine the effect of the dietary treatments on atherosclerosis and the regression of the incidence after garlic feeding by the method of Ludig et al (1992).

RESULTS AND DISCUSSION

Plates 1-20 show the histophotomicrographs of the coronary artery of the heart following the feeding of the experimental feedstuffs without incorporation of garlic and then with incorporation of garlic. For corn meal (T_1), Cowpea (T_2) and Soyabean (T_4) treatments without garlic, it was observed that there was no thickening of the walls of the coronary artery while the arterial lumen remained open. In contrast, for melon (T_3) and cassava (T_5) treatments, the walls of the coronary artery showed thickening with narrowing (stenosis) of the lumen. However, with garlic inclusion, the arterial wall thickening which was hitherto observed in melon and cassava treatments were reduced, and the stenosis reduced as well, leaving fairly open lumen.

TABLE 1: Serum profile of the Chinchilla rabbits fed Corn meal (T_1), Cowpea (T_2), Melon (T_3), Soyabean (T_4) and Cassava (T_5) without Garlic.

S/N	Treatment	Total Lipid (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
1.	Corn meal (T_1), Control	$520.00^{bc}\pm 0.00$	$72.00^{b}\pm 0.00$	$250.00^{c}\pm 0.00$	$380.00^{b}\pm 0.00$	$13.00^{b}\pm 0.00$
2.	Cowpea (T_2)	$565.00^{bc}\pm 0.00$	$72.00^{b}\pm 0.00$	$270.00^{b}\pm 0.00$	$390.00^{b}\pm 30.00$	$13.00^{b}\pm 2.00$
3.	Melon (T_3)	$580.00^{b}\pm 0.00$	$85.00^{a}\pm 0.00$	$340.00^{a}\pm 0.00$	$400.00^{a}\pm 0.00$	$14.00^{a}\pm 0.00$
4.	Soyabean (T_4)	$485.00^{c}\pm 35.00$	$66.50^{c}\pm 11.50$	$275..00^{b}\pm 25.00$	$360.00^{c}\pm 20.00$	$12.50^{c}\pm 1.50$
5.	Cassava meal(T_5)	$670.00^{a}\pm 70.00$	$92.00^{a}\pm 2.00$	$380.00^{a}\pm 60.00$	$455.00^{a}\pm 55.00$	$19.00^{a}\pm 3.00$

a,b,c: Within column, means with different superscripts are significantly ($P<0.05$) different

TABLE 2: Serum profile of the Chinchilla rabbits fed Corn meal (T_1), Cowpea (T_2), Melon (T_3), Soyabean (T_4) and Cassava (T_5) with Garlic.

S/N	Treatment	Total Lipid (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
1.	Corn meal (T_1), Control	$410.00^{b}\pm 13.03$	$52.00^{b}\pm 8.00$	$295.00^{b}\pm 5.00$	$230.00^{b}\pm 20.00$	$7.20^{b}\pm 0.00$
2.	Cowpea (T_2)	$390.00^{b}\pm 0.00$	$54.00^{b}\pm 4.00$	$280.00^{b}\pm 20.00$	$195.00^{b}\pm 5.00$	$5.40^{b}\pm 1.80$
3.	Melon (T_3)	$475.00^{a}\pm 15.00$	$61.00^{a}\pm 11.00$	$304.00^{a}\pm 5.00$	$245.00^{a}\pm 45.00$	$8.10^{a}\pm 2.70$
4.	Soyabean (T_4)	$370.00^{b}\pm 30.00$	$54.00^{b}\pm 0.00$	$285.00^{b}\pm 5.00$	$190.00^{b}\pm 10.00$	$6.30^{b}\pm 9.0$
5.	Cassava meal (T_5)	$450.00^{a}\pm 30.00$	$64.50^{a}\pm 5.50$	$355.00^{a}\pm 15.00$	$230.00^{b}\pm 10.00$	$10.80^{a}\pm 1.80$

a,b: Within column, means with different superscripts are significantly ($P<0.05$) different.

The values for cassava and melon were significantly ($P<0.05$) higher than the values for corn meal, cowpea and soyabean in the parameters measured (Total lipid, Cholesterol, LDL and TG (Table 1). This suggests that cassava and melon are atherogenic. For a feedstuff to be regarded as atherogenic, the level of LDL produced must be higher than that of HDL (Nissen et al, 2006) in addition to the production of high levels of Cholesterol and TG (Keys 1957, Jones, 1974). Melon contains poly-saturated fat and so produced the rise in cholesterol, LDL and TG

(Mayes, 1981), while cassava yields Acetyl – CoA which could then raise the hepatic synthesis of LDL-cholesterol and its plasma levels thereby inducing atherosclerosis. This finding corroborates those reported by Ekine (2007), Bergon and Jacques (1990), Orford et al (2006), FAO report (1977), Oruwari and Ironkwe (1999), Keys (1957) and Jones (1974). In contrast Watson et al (1995) and Assmana *et al.* (1996) reported that because of the LDL oxidation properties, the serum level of HDL should be low while LDL levels are high, for atherosclerosis to occur. Garlic incorporation at the rate of 2og/kg feed produced a reduction in the serum parameters, Total lipid, Cholesterol, HDL, LDL and TG in all the treatments (Table 2) suggesting that garlic inclusion regressed the atherosclerosis produced by the atherogens, cassava and melon. The regression produced by garlic here is due to both its hyperlipidimia lowering and anti-oxidant properties.

TABLE 3: Serum profile of the male and female Chinchilla rabbits fed corn meal (T_1), Cowpea (T_2), melon (T_3), Soyabean (T_4) and Cassava meal (T_5) without Garlic.

S/N	Treatment	Sex	Total Lipid (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
1.	Corn meal (T_1, Control)	M	$520.0^{c}\pm0.00$	$35.0^{d}\pm0.91$	$229.0^{d}\pm0.60$	$310.0^{d}\pm0.18$	$6.60^{d}\pm0.14$
2.	Corn meal (T_1, Control)	F	$555.0^{b}\pm0.87$	$40.2^{c}\pm0.19$	$250.0^{b}\pm0.60$	$335.0^{c}\pm0.18$	$8.0^{b}\pm0.14$
3.	Cowpea (T_2)	M	$506.0^{c}\pm0.14$	$40.0^{c}\pm0.10$	$216.0^{d}\pm0.64$	$330.0^{c}\pm0.56$	$5.0^{d}\pm0.10$
4.	Cowpea (T_2)	F	$520.0^{b}\pm0.14$	$43.2^{b}\pm0.10$	$280.0^{c}\pm0.64$	$361.0^{b}\pm0.42$	$6.0^{d}\pm0.10$
5.	Melon (T_3)	M	$530.0^{c}\pm0.35$	$41.0^{c}\pm0.10$	296.0 ± 0.14	$350.0^{b}\pm4.2$	$6.0^{d}\pm0.20$
6.	Melon (T_3)	F	$565.0^{b}\pm0.14$	$45.2^{b}\pm0.10$	$310.0^{b}\pm0.14$	$400.0^{a}\pm0.54$	$10.1^{a}\pm0.20$
7.	Soyabeam(T_4)	M	$394.0^{d}\pm0.91$	$38.5^{d}\pm0.19$	$220.0^{d}\pm0.25$	$302.0^{d}\pm0.28$	$5.0^{d}\pm0.10$
8.	Soyabean (T_4)	F	$485.0^{c}\pm0.91$	$42.5^{d}+0.91$	$275.0^{c}+0.35$	$340.0^{c}+0.28$	$6.0^{b}+0.10$
9.	Cassava meal (T_5)	M	$579.0^{b}\pm0.91$	$49.6^{c}\pm0.14$	$320.0^{b}\pm0.60$	$377.0^{b}\pm0.78$	$9.0^{a}\pm0.15$
10.	Cassava meal (T_5)	F	$670.0^{a}\pm0.91$	$51.0^{a}\pm0.14$	$380.0^{a}\pm0.60$	$455.0^{a}\pm0.78$	$10.5^{a}\pm0.15$

a,b,c,d: Within column, means with different superscripts are significantly (P<0.05) different.

TABLE 4: Serum profile of the male and female Chinchilla rabbits fed corn meal (T_1), Cowpea (T_2), Melon (T_3), Soyabean (T_4) and Cassava meal (T_5) with Garlic.

S/N	Treatment	Sex	Total Lipid (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
1.	Corn meal (T_1, Control)	M	$430.0^{b}\pm0.0.09$	$30.4^{b}\pm0.06$	$284.0^{b}\pm0.06$	$204.0^{b}\pm0.08$	$3.40^{c}\pm0.03$
2.	Corn meal (T_1, Control)	F	$450.0^{b}\pm0.56$	$33.0^{b}\pm0.025$	$310.0^{b}\pm0.17$	$210.0^{b}\pm0.21$	$6.0^{b}\pm0.06$
3.	Cowpea (T_2)	M	$430.0^{b}\pm0.09$	$30.4^{b}\pm0.06$	$284.0^{b}\pm0.06$	$204.0^{b}\pm0.08$	$3.40^{c}\pm0.03$
4.	Cowpea (T_2)	F	$440.0^{b}\pm0.56$	$32.0^{b}\pm0.25$	$300.0^{b}\pm0.17$	$220.0^{b}\pm0.21$	$6.0^{b}\pm0.06$
5.	Melon (T_3)	M	$430.0^{a}\pm0.09$	$30.4^{b}\pm0.06$	$284.0^{b}\pm0.06$	$204.0^{b}\pm0.08$	$4.40^{b}\pm0.03$
6.	Melon (T_3)	F	$490.0^{a}\pm0.58$	$37.0^{a}\pm0.25$	$340.0^{a}\pm0.17$	$250.0^{a}\pm0.21$	$8.0^{a}\pm0.06$
7.	Soyabeam(T_4)	M	$420.0^{b}\pm0.09$	$30.4^{b}\pm0.06$	$284.0^{b}\pm0.06$	$204.0^{b}\pm0.08$	$3.40^{c}\pm0.06$
8.	Soyabean (T_4)	F	$440.0^{b}\pm0.56$	$32.0^{b}+0.25$	$310.0^{b}+0.17$	$210.0^{b}+0.21$	$5.0^{b}+0.06$
9.	Cassava meal (T_5)	M	$430.0^{b}\pm0.09$	$30.4^{b}\pm0.06$	$284.0^{b}\pm0.06$	$204.0^{b}\pm0.08$	$7.40^{a}\pm0.03$
10.	Cassava meal (T_5)	F	$490.0^{a}\pm0.56$	$37.0^{a}\pm0.25$	$345.0^{a}\pm0.017$	$265.0^{a}\pm0.21$	$8.0^{a}\pm0.06$

a,b,c: Within column, means with different superscripts are significantly (P<0.05) different.

When the blocking (sex) effect was considered there was significant difference (P<0.05) in the levels of Cholesterol, HDL, LDL, TG and Total lipids between the male and the female rabbits with the female rabbits showing more lowering of these values (Tables 4,5). This finding is not in consonance with those obtained by Lusis (2000) and Ekine (2007). The findings of this study on garlic feeding also corroborate the findings of Mirhadi et al (1993), Jain (1993), Chang et al (1977), Kamanna et al (1980), Mand et al (1984), Schwartz et al (2001), Gebhardt (1991), Rotzsh (2001), Oruwari et al (1999), Berthold et al (1998), Mathew et al (1996), Eilat (1995), Shoetan et al (1984), Ishmail et al (1999), Heinle et al (1994), Kosciency (1999), Yeh et al (1994), Orekhov (1996), Mirhadi et al (1986) on rabbits and also that of Oruwari (2006) on Japanese quails. To further demonstrate the effect of the experimental feedstuffs, histophotomicrographic characterization of the coronary arteries of the hearts of the rabbits was carried out (Plates 1-20) and their results compared with those of the lipid metabolites, Cholesterol, HDL, LDL and TG. Cowpea caused a slight thickening of the walls of the coronary arteries and an accumulation of plagues in the lumen. These were regressed by garlic feeding. Cassava and melon caused a pronounced thickening (atheromatous plagues) of the wall of the coronary artery indicated by extruded myofibrils (hypertrophy of the intimal layer). These conditions were also regressed by garlic feeding. This corroborates the work of Ekine (2007) who observed atherosclerotic lesions and plagues in the carotid and coronary arteries of the

Japanese quail fed cassava corn meal diets and that of Oruwari and Ironkwe (2000) who observed similar lesions and plagues in the carotid arteries of rabbits fed palm oil, groundnut oil and corn mean diets. Soyabean did not cause thickening of the intima of the coronary arteries and there was no blocking or occlusion of the lumen. This suggests that soyabean way contain a substance that has anti-cholesterolic effect. Garlic regressed the slight thickening mentioned above which suggests that soyabean did not produce atherosclerosis and that soyabean is not an atherogen.

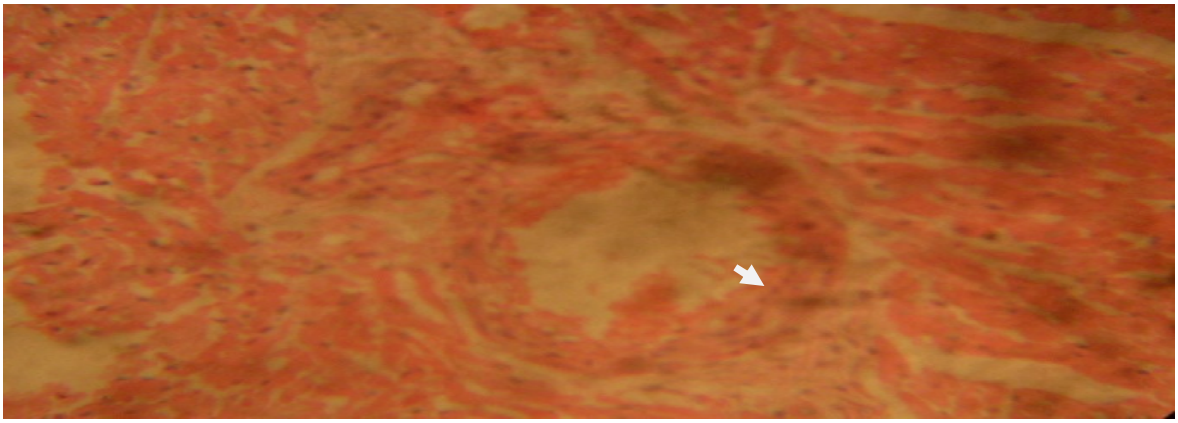

PLATE 1: H&E X4OO Heart . coronary heart vessel with thick wall (see arrow)

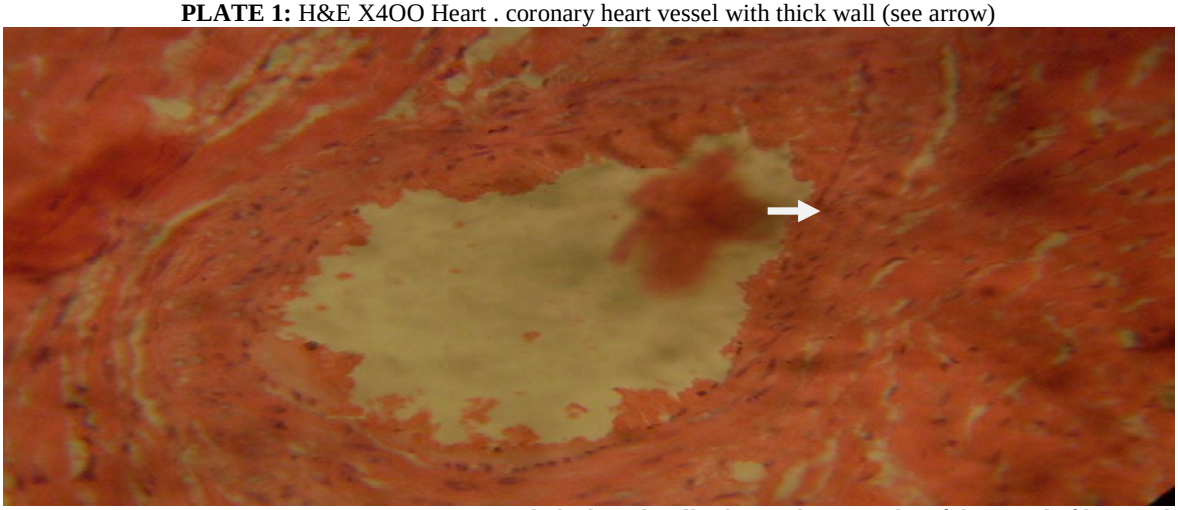

PLATE 2: H&E X4OO HEART. Coronary artery with thickened wall. There is hypertrophy of the muscle fibres in the media (arrow).

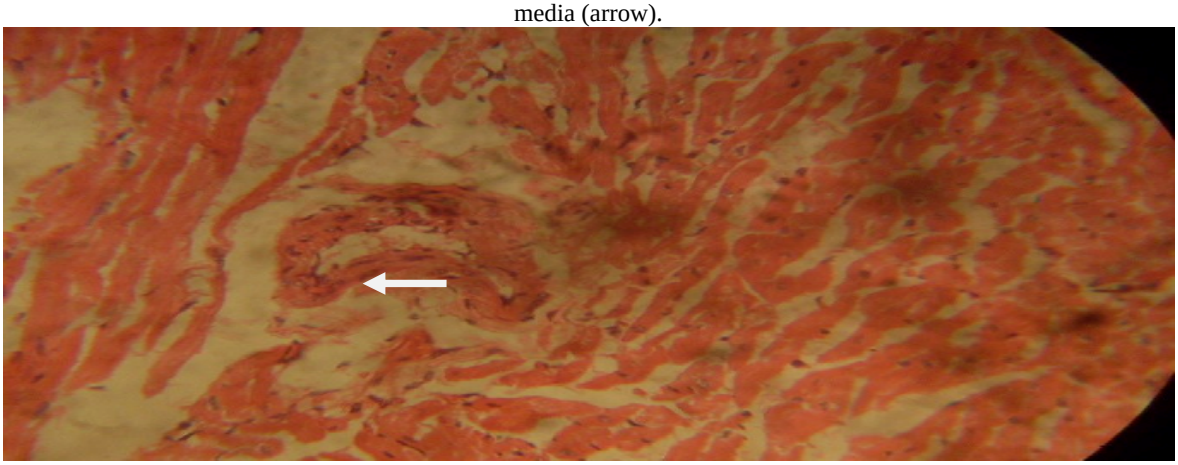

PLATE 3: H&E X400 HEART. Coronary artery with thickened wall. There is hypertrophy of the muscle fibres in the media (arrow).

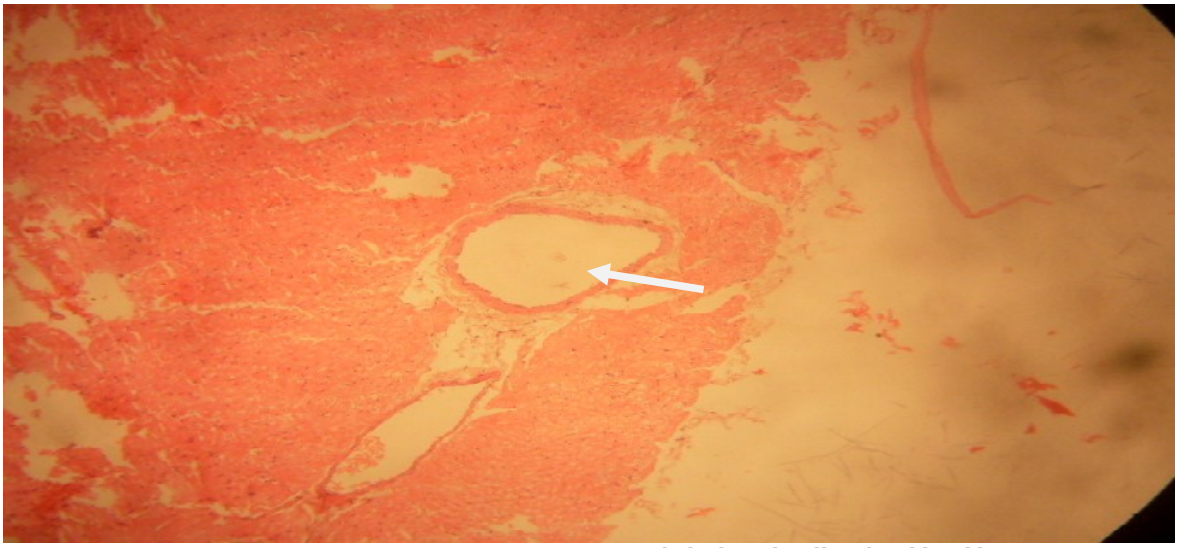

PLATE 4: H&E X400 HEART. Coronary artery with thickened wall with a dilated lumen.

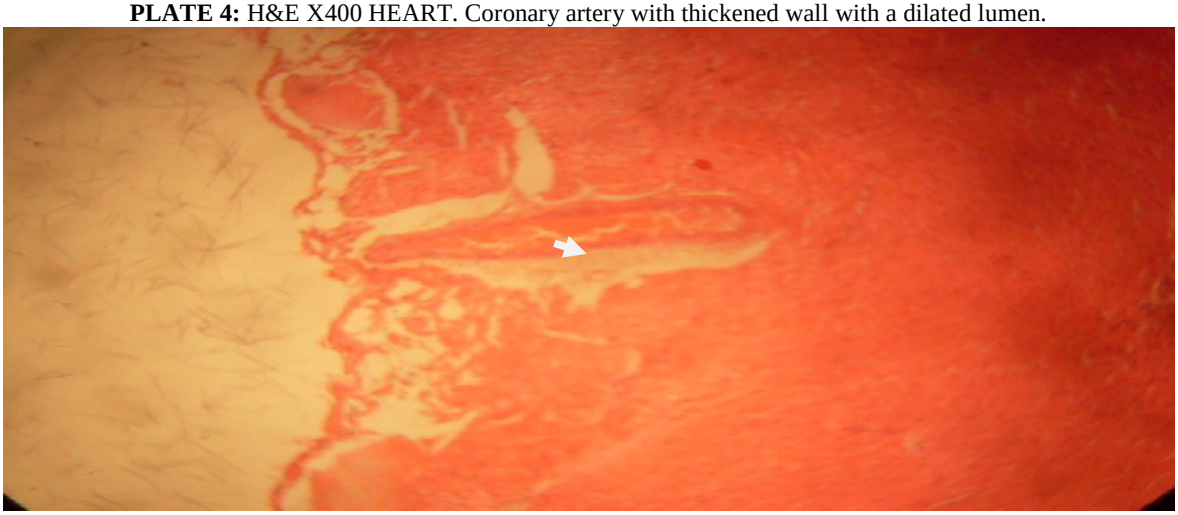

PLATE 5: H&E X200 HEART. Coronary artery branch that is patent and filled with red blood cells.

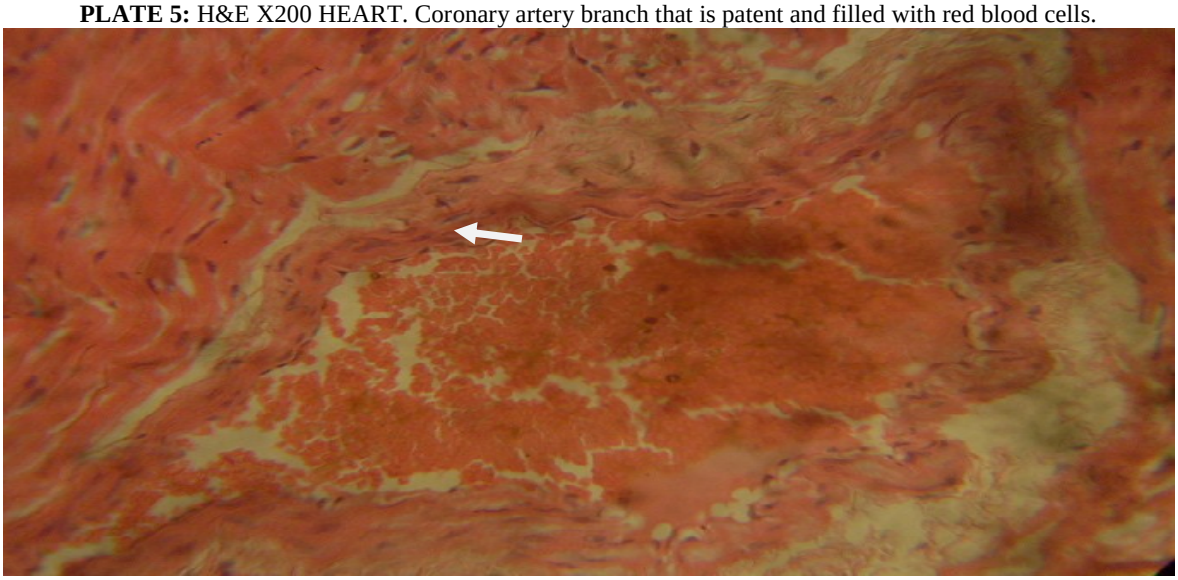

PLATE 6: H&E X400 HEART. Coronary artery with a dilated lumen and thickened wall.

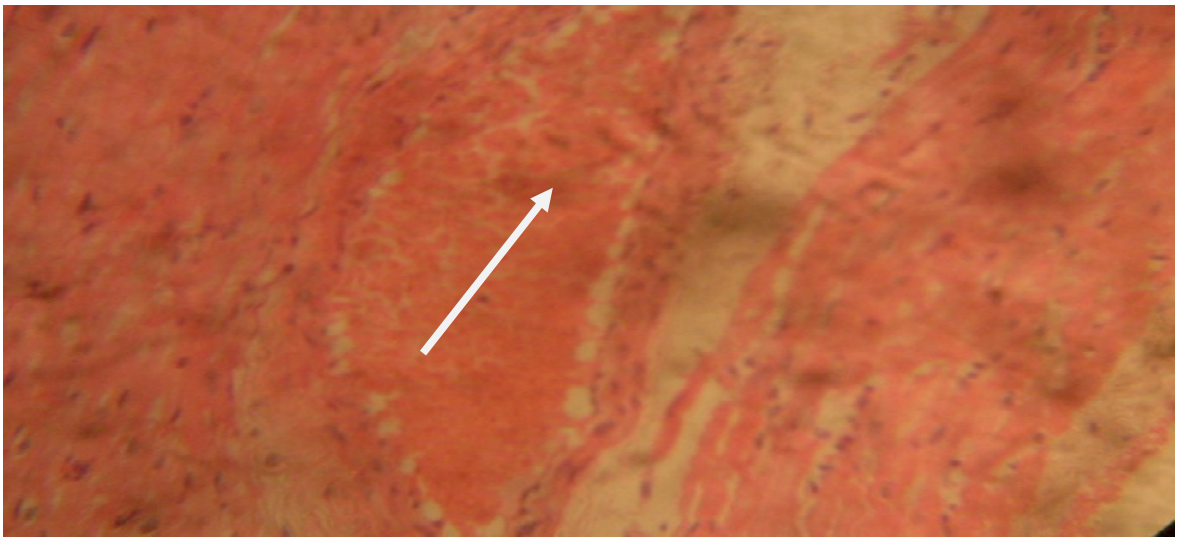

PLATE 7: H&E X400 HEART. Coronary artery with a dilated lumen and thickened wall.

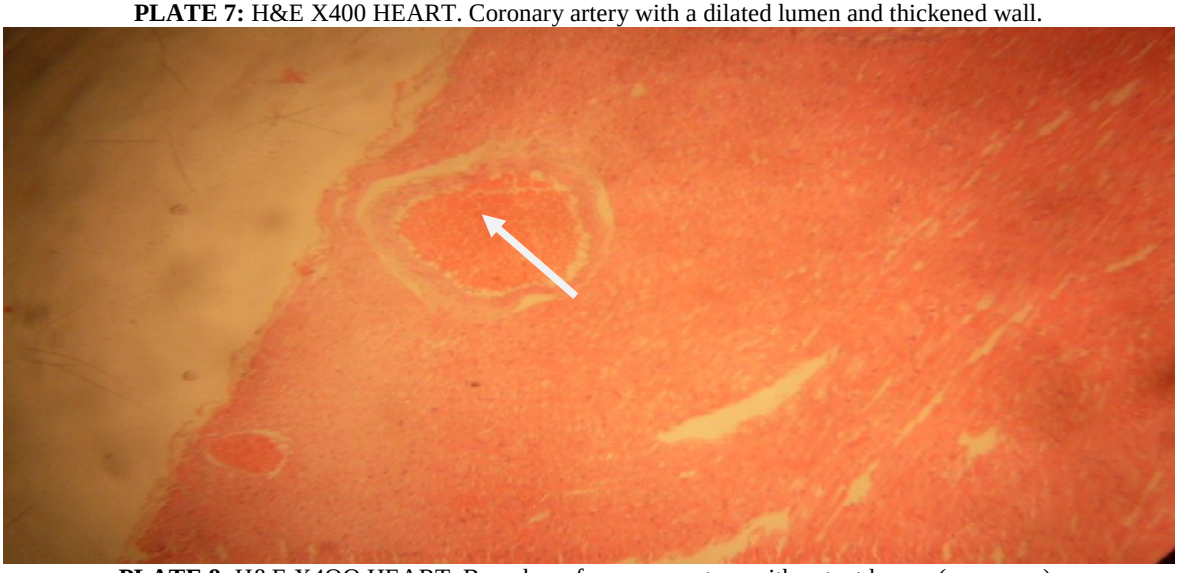

PLATE 8: H&E X4OO HEART. Branches of coronary artery with patent lumen (see arrow).

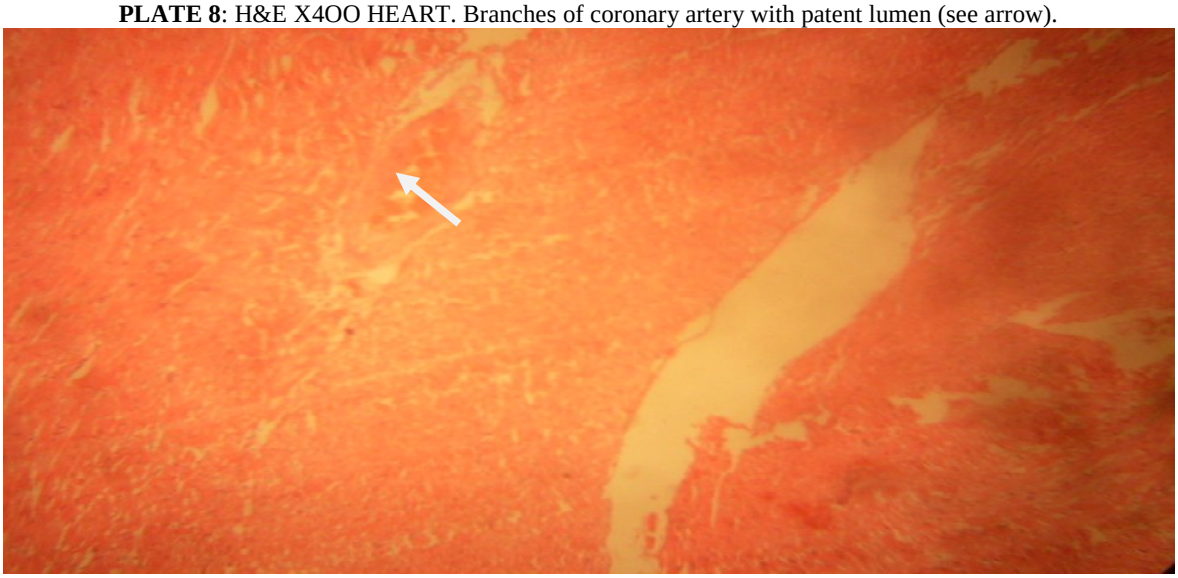

PLATE 9: H&E X2OO HEART. Branches of coronary artery with patent lumen.

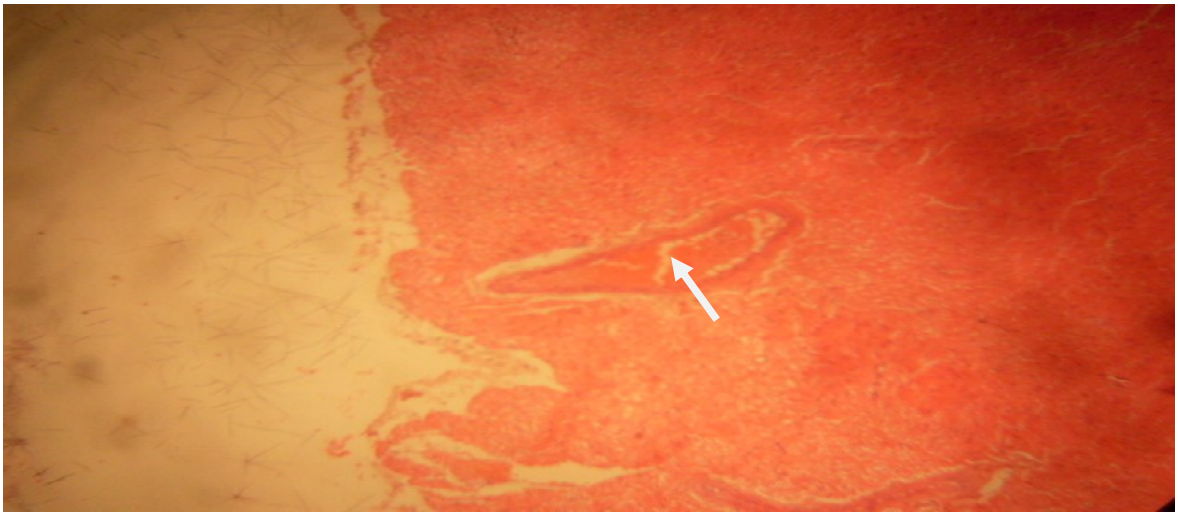

PLATE 10: H&E X2OO HEART. Branches of coronary artery with patent lumen (see arrow).

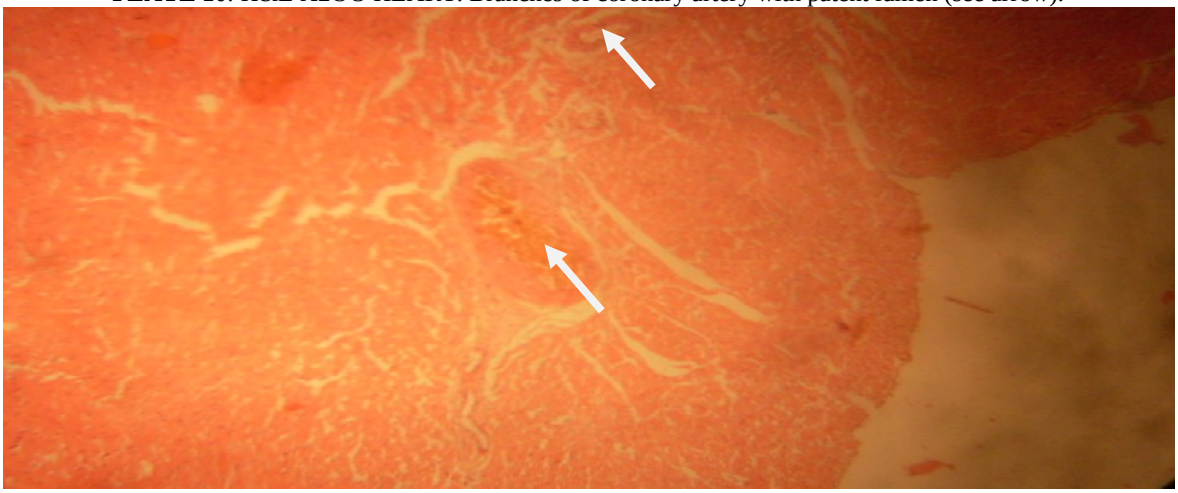

PLATE 11: H&E X4OO HEART. Branches of coronary artery with patent lumen (see arrows).

PLATE12 : H&E X2OO HEART. Branch of coronary artery with patent lumen (see arrow).

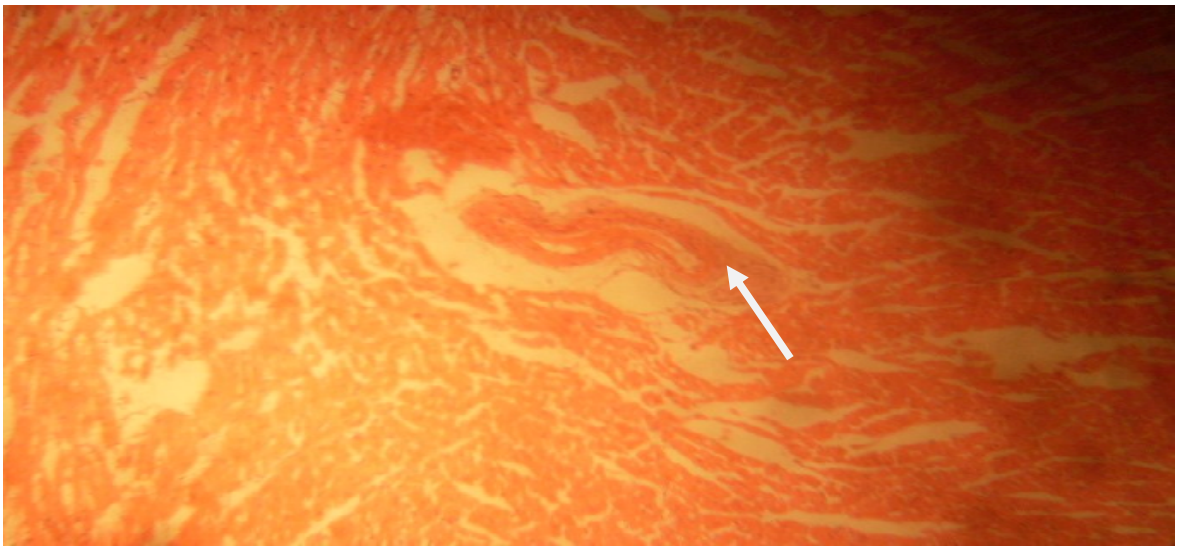

PLATE 13: H&E X4OO HEART. Branch of coronary artery with a thickened wall(see arrow).

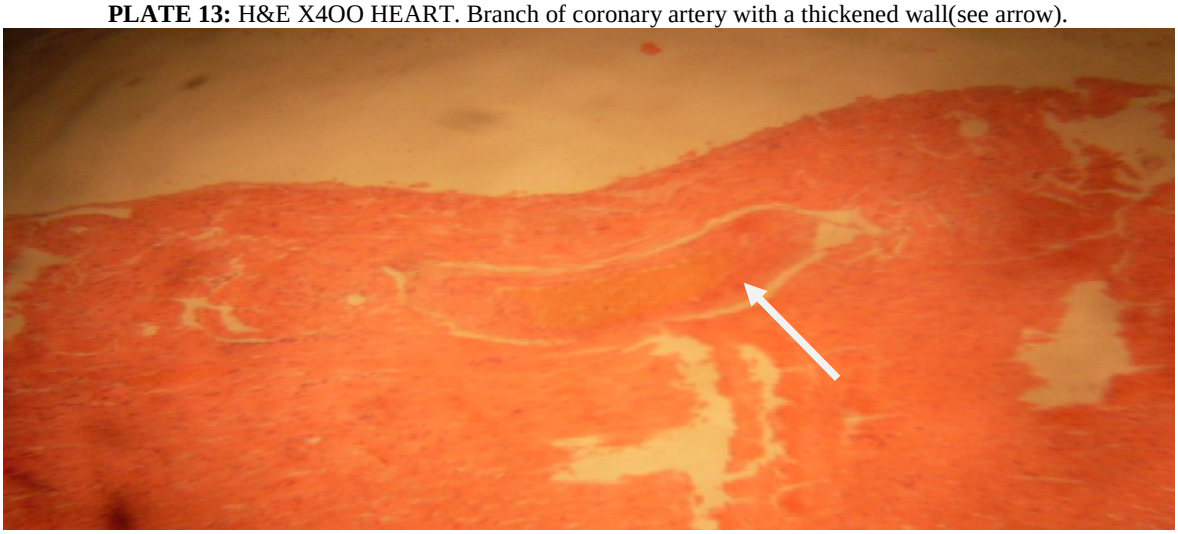

PLATE 14: H&E X4OO HEART. Branch of coronary artery with patent lumen and a thickened wall (see arrow).

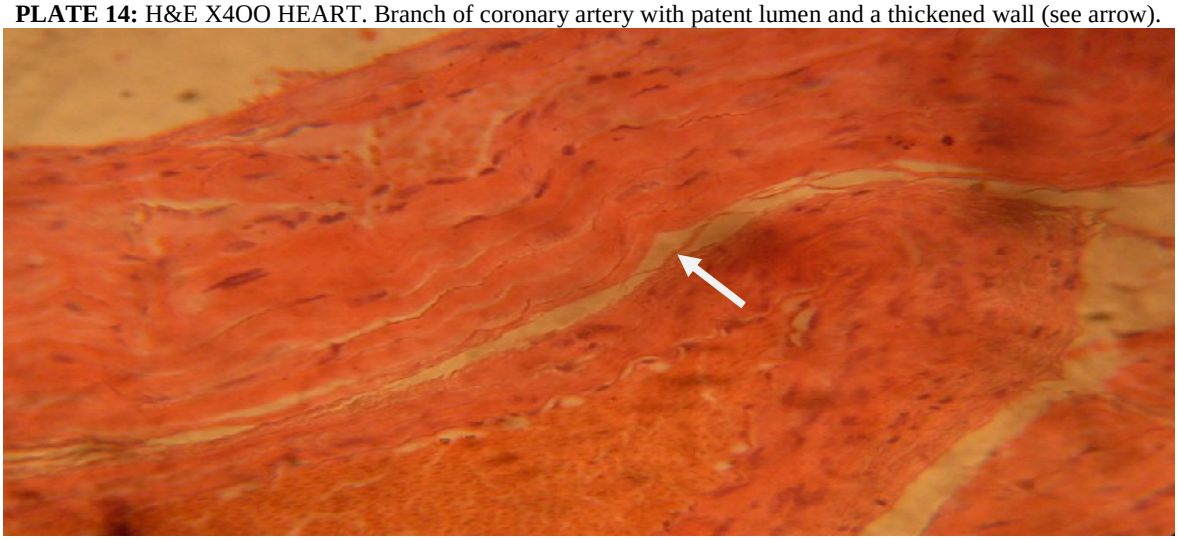

PLATE 15: H&E X4OO HEART. Branch of coronary artery with patent lumen and thickened media (see arrow).

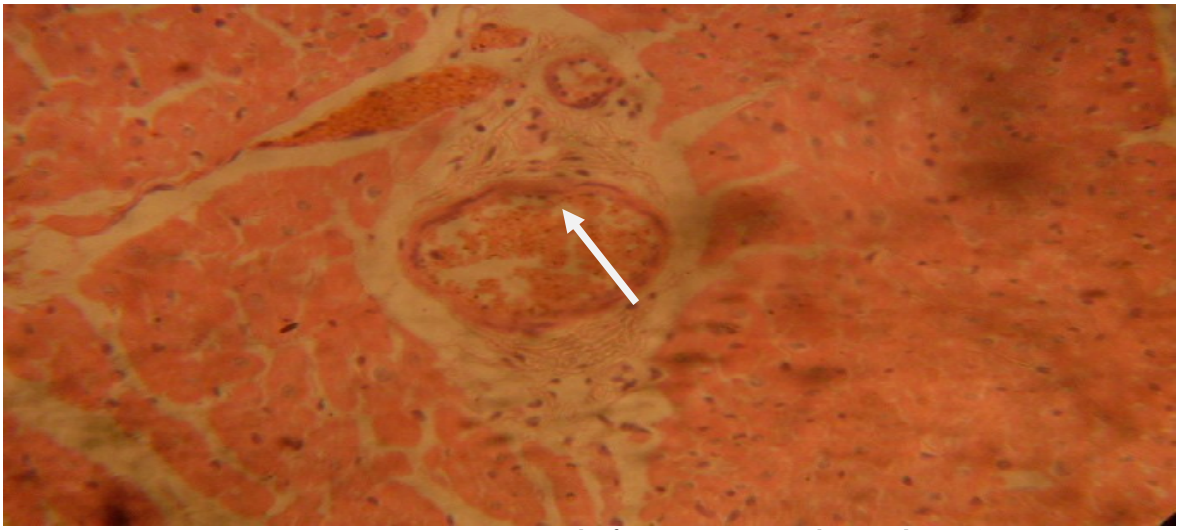

PLATE 16 : H&E X4OO HEART. Branch of coronary artery with patent lumen (see arrow).

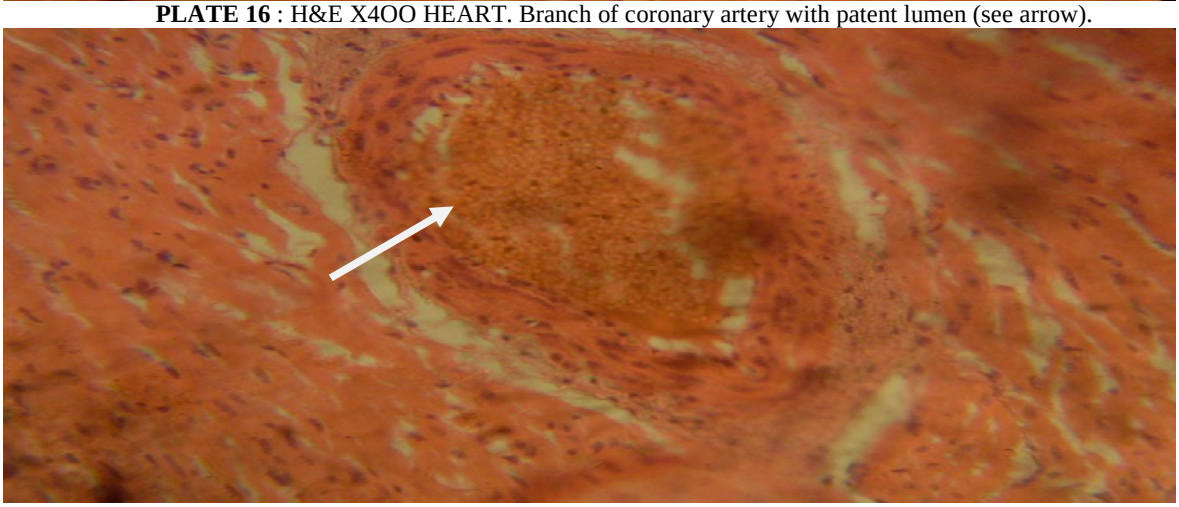

PLATE 17: H&E X4OO HEART. Branch coronary artery with patent lumen and hypertrophy of the media myocytes (see arrow).

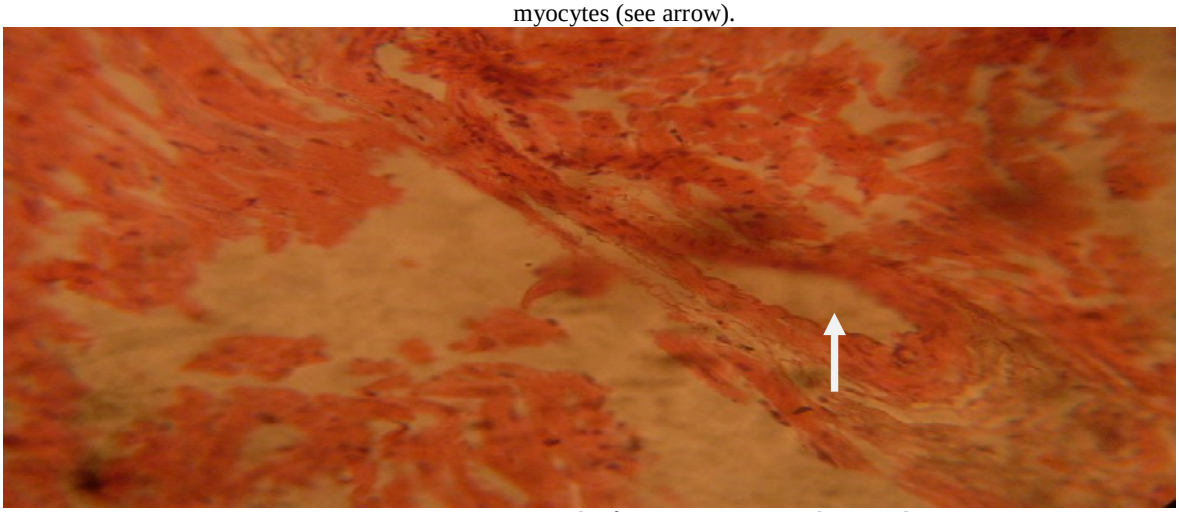

PLATE 18 : H&E X4OO HEART. Branch of coronary artery with patent lumen (see arrow).

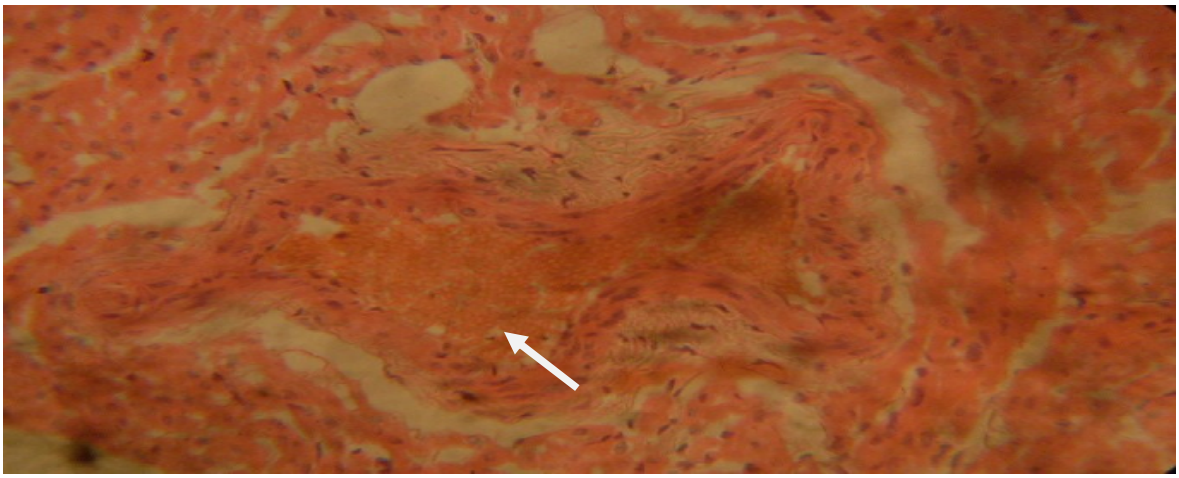

PLATE 19: H&E X4OO HEART. Branch of coronary artery with patent lumen and hypertrophy of the media (see arrow).

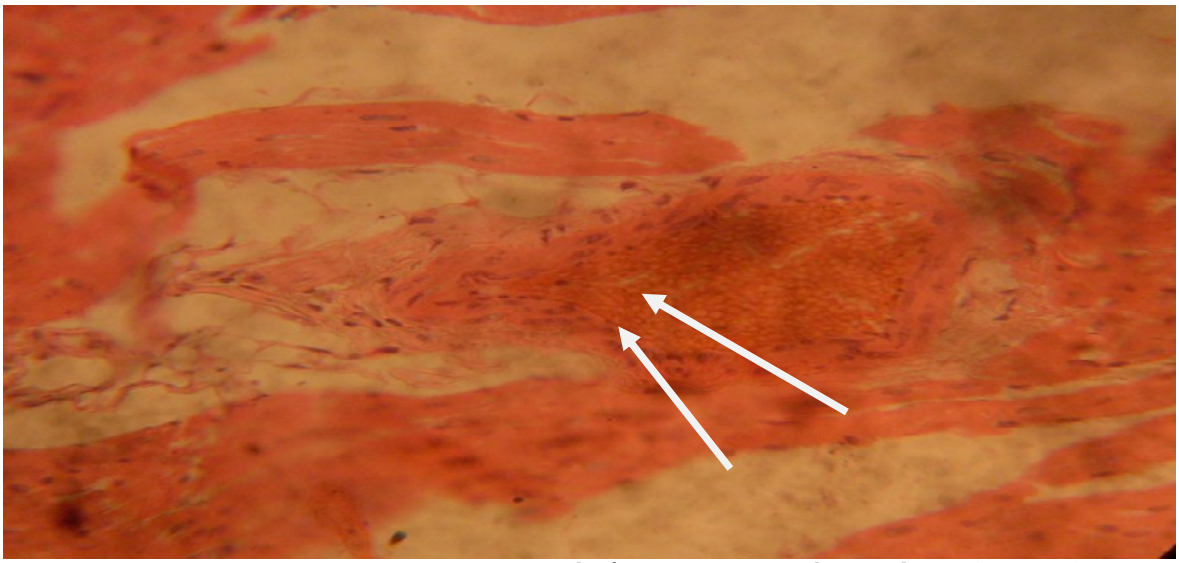

PLATE 20: H&E X4OO HEART. Branch of coronary artery with patent lumen (see arrow).

Cassava meal (garri) caused a thickening of the wall of the coronary artery which was demonstrated as the myocytes of the intima seem extruded and the hypertrophy of the intimal wall clearly shown. It showed that cassava (garri) caused atherosclerosis and that cassava is atherogenic. However, garlic feeding caused a regression of the atherosclerosis produced by cassava. The finding in this study was also consistent with those of Reidy et al (1992) and Ross (1999) who reported that an atherosclerotic lesion starts when the endothelium is injured, leading to endothelial plague formation. The endothelial injury causes oxidation of the LDL – Cholesterol leading to OX-LDL Cholesterol formation which causes further injury and thickening of the arterial wall (intima). Plates 6,9,10, 19 and 20 are indicative of atherosclerosis followed by formation of atherosclerotic plagues with the lumen showing lipid-engorged leukocytes (foam cells) which are being pushed out into the arterial blood vessel lumen by smooth cells (Hachinskin, 1992, Clinton and Libby, 1992). The feeding trial showed that the thickening was more in the females that in the males corroborating the observations of Ekine (2007) and Ironkwe (2002), who established that the push of atherosclerotic plagues into the vessel lumen was more in female Japanese quails than in the males.

CONCLUSION

In this study, cassava and melon have been shown to be atherogenic. This was demonstrated by the rise in serum lipids, namely, Total lipids, Cholesterol, HDL, LDL and TG which was produced by the feeding of these feedstuffs in rabbits, complimented by the demonstration of endothelial injury and thickening of the coronary arterial walls by histophotomicrographic characterization. In all, the erstwhile atherogenesis and, or atherosclerosis was regressed by garlic feeding.

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